FINAL REPORT

Biotechnology Risk Assessment Research Grants Program
Award No. 95-33120-1876
9502381 Effect of Sequence Similarity on Recombination Frequency

Peter Palukaitis
Department of Plant Pathology
Cornell University

Aims:

1. To determine under what conditions and with what frequency recombination occurs in transgenic plants, between the transgene mRNAs and defective viruses of increasing sequence dissimilarity;
2. To examine the effect of transgene mRNA level on the recombination frequency;
3. To determine the frequency and sites of recombination between two defective viruse of increasing dissimilarity in sequence, in non-transformed plants;
4. To compare these data with the frequency (and sites) of recombination between a defective virus and transgene mRNAs.

Results:

1. A variety of defective viruses were generated using strains of cucumber mosaic virus (CMV), showing 1-2%, 9%, or 25% differences in sequence from the viral transgene, as well as a strain of tomato aspermy virus (TAV), which differs by 35% in sequence from the CMV strains. These viruses were passaged in transgenic plants under conditions where genetic complementation would occur, to determine whether recombination occurred between the expressed transgene and the defective viral RNAs, and with what frequency. In the case of the various CMV strains, the frequency of recovered recombinants was very low (ca. 1%), irrespective of the level of sequence divergence from the transgene (i.e., 1-2%, 9% or 25%). No recombinants were detected between TAV and the CMV transgene. The nature of the recombinants explained the unexpected observations: Recombination occurred in the intergenic region between the genes encoding the movement protein and the capsid protein. This region contained highly conserved sequence elements and did not show the same level of sequence divergence as did the flanking sequence in the two genes. In the case of TAV, there was not the same degree of conservation of the sequence elements in the intergenic region. Thus, it is not the overall sequence similarity that dictates the frequency of recombination, but rather the sequence similarity in specific regions that are the "hot spots" for recombination. Also, recombination occurred even though the transgene did not contain the viral 3' end non-translated region.

2. Different transgenic lines expressing different levels of the transgene did not show any significant difference in recombination frequency. This might be expected, since recombination occurred from the inoculated virus (donor sequence) to the transgene (recipient sequence), with the former in large excess over the latter. Thus, the kinetics of the reaction were effectively pseudo-first order. However, it should be noted that the differences in transgene expression level were 2-10 fold, and not 10-100 fold, as might occur using transgenes which have undergone post-transcriptional gene silencing.

3. When two defective viral RNAs were co-inoculated to non-transgenic plants, recombination occurred rapidly and in most of the plants inoculated. As before, recombination did not occur between a defective CMV RNA and a defective TAV RNA. Also, the sites of recombination were similar to what was observed above, indicating that recombination was occurring by a similar mechanism. However, the high concentration of the two components involved in the recombination, plus the fact that recombination could occur from either partner as a donor, means that completely different recombination kinetics are involved. Thus, the frequency of recombination occurring between dysfunctional viruses cannot be used as a guide to the frequency of recombination likely to occur between a defective virus and a transgene. By contrast, the absence or very low frequency of detectable recombination between defective, unrelated virus would indicate that recombination between a transgene of one virus and an unrelated virus would occur even less frequently. That is, in transgenic plants, such recombination events would be less likely to occur by at least two orders of magnitude than between two unrelated viruses.

4. Given the difficulty of naturally obtaining a recombinant virus formed from two unrelated virus [e.g. CMV and tobacco mosaic virus (TMV)], we generated such an artificial virus using recombinant DNA technology, and assessed this virus (T/CMV-1) for any competitive edge it might have over the parental viruses. T/CMV-1 was unable to move locally in cucurbit hosts or long-distance in tobacco. [TMV can move cell-to-cell in its hosts in the absence of capsid protein (CP), but requires CP for rapid, long-distance movement via the vascular system. T/CMV-1 consisted of TMV containing the CMV CP in place of its own.] In *Nicotiana benthamiana* and *N. clevelandii* (species highly permissive to virus movement), T/CMV- 1 could move long-distance, but much more slowly than wildtype TMV, indicating that the hybrid virus was moving cell-to-cell up the stem and not via the vascular system. Nevertheless, in the systemically infected leaves, the level of CMV CP was similar to that produced by wildtype CMV and CMV-like particles could be isolated. The yield of virus particles was about 10% of that obtained from CMV-infected plants, and they were either poorly or not infectious, depending on the preparation. In the former case, the preparations could also be transmitted by aphids (the vector of CMV, but not TMV), albeit poorly and sporadically. Such particles contained trace amounts of full-length T/CMV-1 genomic RNA (6.5kb), whereas the rest of the RNA encapsidated was in the size range of the CMV RNAs (1-3.5kb). Thus, even if the recombinant virus could be formed between these two unrelated viruses in such a way that the entire CP of CMV was expressed from an intact subgenomic promoter on TMV, it would not have any selective advantage over either parental virus, but rather, would be severely debilitated, because of restricted virus movement and packaging limitations of the CMV CP.

5. CP mutants were generated to determine how important an intact CP was for virus movement. One mutant with a 15 amino acid deletion showed no delay in systemic infection, but showed altered pathology. A second mutant with a 26 amino acid deletion was not able to either form virus particles or move long-distance in tobacco, but could move slowly long distance in *N. clevelandii* and *N. benthamiana*, although again outside the vascular system. The third mutant with virtually the entire CP gene deleted was not able to move even cell-to-cell in any hosts tested. Thus, while the CP was necessary for cell-to-cell movement, the ability to form virus particles was not necessary for cell-to-cell movement, but was necessary for vascular-dependent, long-distance movement. These mutant viruses were not used in recombination studies with transgenic plants expressing the C\1V CP, to examine the

effect of different selection pressures on the recombination frequency to regenerate a wildtype virus, since the only transgenic plants available were post-transcriptionally silenced for CP gene expression. While the latter situation may more accurately have mimicked the situation in the field, it would not have allowed an assessment of recombination frequencies to be made in the greenhouse. The scale of inoculum preparation required to make such an assessment in the field would have been cost prohibitive.

Publications:

Haudenshield, J.S. and Palukaitis, P. 1998. Diversity among isolates of squash mosaic virus. Journal of General Virology 79:2331-2341.

Kaplan, I.B. and Palukaitis, P. 1998. Characterization of cucumber mosaic virus. VI. Generation of deletions in defective RNA 3s during passage in transgenic tobacco expressing the 3a gene. Virology 251:279-287.

Kaplan, I.B., Zhang, L. and Palukaitis, P. 1998. Characterization of cucumber mosaic virus. V. Cell-to-cell movement requires capsid protein but not virions. Virology 246:221-231.